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Eat Your Carrots! T Cells Are RARing to Go

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In this issue of *Immunity*, Hall et al. (2011) show that vitamin A and its metabolites play a central role in regulating adaptive immunity by promoting the development of both inflammatory and regulatory T cell responses.

Vitamin A is an essential fat-soluble nutrient that is required for the development of many organs and tissues. It is converted into several metabolically active derivatives, including retinoic acid (RA). RA is a potent regulatory molecule that controls gene expression through RA receptors (RARs), members of the nuclear hormone receptor family that activate the transcription of specific target genes. The RA-RAR signaling machinery represents an evolutionarily ancient gene regulatory pathway, which is conserved in all vertebrates and invertebrate chordates. It appears to have evolved as a core regulatory network that governs cell fate specification during developmental patterning. In vertebrates, RA also plays important roles in the development of specific immunity, directing immunoglobulin class switching in B cells and inducing gut homing receptors on B and T lymphocytes. However, until relatively recently there was little known about how RA impacts T cell development.

In 2007, a raft of landmark papers from several laboratories revealed a critical role for RA in directing intestinal $CD4^+$ T cell development (Benson et al., 2007; Coombes et al., 2007; Denning et al., 2007; Mucida et al., 2007; Sun et al., 2007). According to these initial studies, this effect was restricted to a particular subset of $CD4^+$ T cells. $CD4^+$ T cells can develop along one of several distinct pathways that dictate the type of immune response that ensues. T helper type 1 (Th1) and Th17 cell developmental subsets are associated with aggressive proinflammatory responses to infection, whereas T regulatory (Treg) cells constitute a distinct developmental subset that is associated with immune responses that prevent or dampen inflammation. Each of the studies from 2007 showed that RA signaling triggered intestinal Treg cell development. A specific subset of intestinal dendritic cells (DCs) produced RA, which synergized with the cytokine TGF-β to induce Foxp3 in naive $CD4^+$ T cells, promoting differentiation along the Treg cell pathway (Coombes et al., 2007; Sun et al., 2007). At the same time, other studies revealed that RA inhibited Th17 cell development, leading to the idea of RA as an anti-inflammatory factor that promotes intestinal Treg cell differentiation at the expense of Th17 cell development (Elias et al., 2008; Mucida et al., 2007).

In this issue of *Immunity*, Hall et al. (2011) challenge the idea that RA is an anti-inflammatory factor that restricts T cell differentiation to the Treg cell pathway. Instead, the authors show that RA plays a much broader role in controlling $CD4^+$ T cell fate (Figure 1). To investigate the role of vitamin A and its metabolites in $CD4^+$ T cell responses during infection, Hall et al. put mice on a diet lacking vitamin A. They then provoked inflammation by using *Toxoplasma gondii*, an intracellular protozoan parasite that normally enters the body through the gastrointestinal tract, eliciting robust mucosal inflammation and a strong systemic Th1 cell response. When Hall et al. examined $CD4^+$ T cells after oral infection with *T. gondii*, they observed that mice on a vitamin A-deficient diet had a marked reduction in Th1 cell

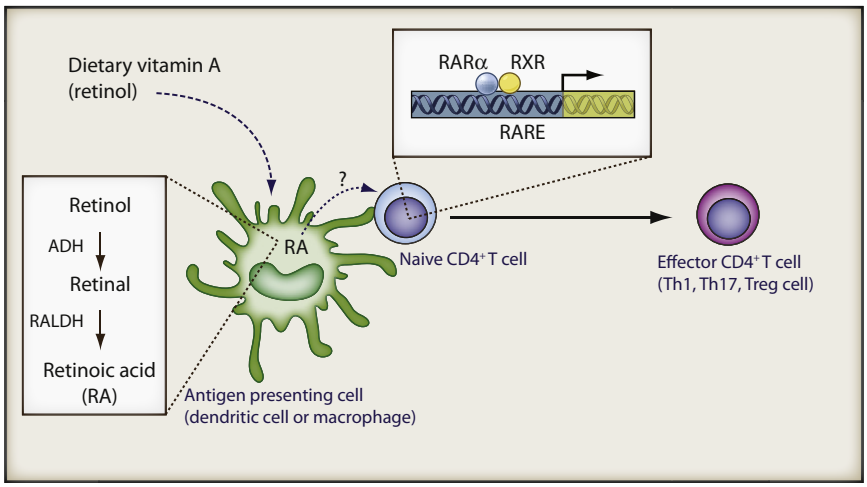

Figure 1. RA Promotes Multiple CD4$^+$ T Cell Effector Responses through RARα Activation
RA was previously thought to be a critical switch factor that promotes Treg cell differentiation but inhibits Th1 and Th17 cell differentiation. However, Hall et al. (2011) demonstrate that RA is also required for Th1 and Th17 cell responses to infection and vaccination and is therefore a broad regulator of CD4$^+$ T cell responses. RA is delivered to T cells, where it activates RA receptor alpha (RARα), a member of the nuclear hormone receptor family. RARα partners with members of the retinoid X receptor (RXR) family to activate transcription of genes that have RA response elements (RAREs) in their promoters. This promotes differentiation of naive T cells to fully functional effector cells that can drive proinflammatory responses to infection. The likely source of RA is antigen-presenting cells, such as dendritic cells or macrophages. These cells express enzymes, including alcohol dehydrogenases (ADHs) and retinaldehyde dehydrogenases (RALDHs), that convert vitamin A (retinol) to RA. The mechanism of RA transfer between dendritic cells and T cells remains unknown.

numbers in their intestines and spleens. Moreover, the vitamin A-deficient mice were infected with more *T. gondii* parasites at both mucosal and systemic sites, and this difference was observed regardless of whether the pathogen was inoculated via the natural oral route or injected systemically. Importantly, robust CD4$^+$ T cell immunity to *T. gondii* was restored by simply feeding RA to the vitamin A-deficient mice. These results indicate that RA is critically important for the T cell response to *T. gondii* and strongly challenge the model of RA as an anti-inflammatory factor whose effects are restricted to mucosal tissues. Instead, the findings show that RA signaling is sustained during infection and promotes the development of an inflammatory Th1 cell response at both mucosal and systemic sites.

Hall et al. also probed RA involvement in T cell responses to vaccination. Employing ovalbumin as a model antigen, they found that oral inoculation of vitamin A-deficient mice fails to elicit Th1 and Th17 cell responses that occur in mice on a normal diet. Thus, vitamin A is essential for both Th1 and Th17 cell responses to oral vaccination. As with *T. gondii* challenge, simply feeding RA to vitamin A-deficient mice restored their ability to respond to mucosal vaccination. As discussed below, this finding has tremendous implications for designing vaccination strategies in human populations, particularly in the face of malnutrition.

The profound impact of RA on CD4$^+$ T cell immunity raises the question of how RA imparts its effects to T cells. In other physiological systems RA activates specific gene expression programs through RARs. Moreover, RA is known to drive Treg cell differentiation by activating RARα, one of the three members of the RAR family (Nolting et al., 2009). Similarly, Hall et al. found that mice lacking RARα showed severely impaired Th1 cell responses to oral vaccination with ovalbumin, establishing that RARα also activates pro-inflammatory CD4$^+$ T responses. RARα triggered signal transduction events downstream of the T cell receptor that direct T cell activation, arguing that RA-RARα signaling occurs within the T cells themselves rather than through an indirect route. Thus, it seems that T cells have co-opted the evolutionarily ancient RA-RAR signaling machinery to govern regulatory networks that are fundamental for their development.

The finding that RA directs proinflammatory T cell responses is in direct conflict with earlier reports, which showed that RA strongly inhibited Th17 cell polarization in vitro (Elias et al., 2008; Mucida et al., 2007). What accounts for this discrepancy? Hall et al. suggest the importance of two factors. First, RA concentration appears to be critical in determining whether Th17 cell differentiation is activated, with lower RA concentrations (1–10 nM) stimulating and higher concentrations (>100 nM) suppressing Th17 cell responses (Wang et al., 2010). Second, the presence of microbial antigens may be critical in determining the outcome of RA stimulation of naive T cells. For example, the Toll-like receptor 5 ligand flagellin stimulates intestinal DCs to both produce RA and induce Th17 cell differentiation (Uematsu et al., 2008). However, it will be important to investigate whether there are in vivo situations in which RA represses Th17 cell development and to determine under what conditions RA activates proinflammatory versus anti-inflammatory T cell responses.

A major remaining question concerns the source of the RA that stimulates inflammatory T cell responses in vivo. T cell development is typically triggered by interactions with antigen-presenting cells (APCs) that involve direct cell-cell contact that is co-incident with T cell receptor engagement. Two lines of evidence suggest that APCs such as DCs might supply the RA that promotes Th1 cell and Th17 cell differentiation. First, intestinal DCs express enzymes that convert retinol to RA (Coombes et al., 2007). Second, DCs supply the RA that induces intestinal Treg cell development (Coombes et al., 2007; Sun et al., 2007), suggesting that they may perform a similar function in proinflammatory T cell development. It is not yet clear whether systemic DCs harbor RA generating machinery or whether RA production is restricted to mucosal DCs. However, given that both mucosal and systemic immune responses to *T. gondii* require RA, it seems likely that RA production may be a general feature of DCs.

Although it is currently not clear how RA is transported between neighboring cells, its lipid solubility suggests that it may be able to permeate adjacent membranes. We speculate that RA may act as a general signal that contact has been achieved between an APC and a T cell. Passage of RA from the APC to the T cell could act as a "molecular handshake" to signal stable cell-cell contact, setting the stage

for activation of specific T cell developmental programs. In this regard, RA signaling in T cell development may be similar to the induction of developmental patterning, where RA signals are frequently passed between adjacent cells, triggering gene expression programs that convey positional information.

The findings of the Hall et al. study have profound implications for public health. First, they indicate that adequate vitamin A intake is critical for productive T cell responses to infection. This idea is supported by the markedly increased susceptibility to infection that is a hallmark of vitamin A deficiency in humans. Second, they indicate that mucosal vaccination in the face of poor nutrition is likely to yield suboptimal results. The fact that RA feeding restored robust $CD4^+$ T cell responses in vitamin A-deficient mice suggests vitamin A or RA supplementation as a strategy to increase the efficacy of vaccination. In other words, you should eat your carrots.

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